6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring acrolein in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify acrolein. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect acrolein in environmental samples are the methods approved by federal agencies such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by a trade association such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Data regarding the analytical methods used in the determination of acrolein in biological samples are limited. Boor and Ansari (1986) developed a method capable of detecting nanogram (ng) quantities of acrolein in biological samples. In this method, a derivatizing agent, 2,4-dinitrophenylhydrazine (DNP), is incubated with liver or kidney homogenate for a short period of time. The acrolein-DNP adduct is then extracted from the sample with chloroform. Analysis for acrolein is accomplished by elution on a reverse phase column using high performance liquid chromatography (HPLC), and detection of the adduct by UV absorbency. Interferences due to the coincidental elution of DNP adducts of ketones or aldehydes other than acrolein are not ruled out by this method of analysis.

Derivatization methods for the measurement of acrolein levels in biological media should be used with caution based upon data for analysis for acrolein in aqueous solutions (Kissel et al. 1978). Methods that utilized derivatives (DNP and 7-hydroxyquinoline) combined with calorimetric *or* fluorimetric detection were not specific for acrolein and consistently did not correlate with those obtained from bioassays. Certain direct methods of detection (nuclear magnetic resonance (NMR), fluorescence, and differential pulse polarography) gave the best correlation to the bioassay results (see discussion of analysis of environmental samples in Section 6.2).

Alarcon (1976) developed a method for quantitifying 3-hydroxypropylmercapturic acid (MCA), a known metabolite of acrolein, in urine. This method involves acidification of the urine to convert MCA to S-(3-hydroxypropyl)-L-cysteine. The amount of S-(3-hydroxypropyl)-L-cysteine can then be quantitated using an automated amino acid analyzer.

Acrolein in biological samples can be detected using a technique developed to determine volatile organic compounds in fish (Hiatt 1983). In this method, homogenized fish are transferred to a special vessel and the

volatile organic compounds are vacuum distilled into a cold trap. The sample is introduced into a cryogenic focusing trap, and the acrolein in the sample is detected by a mass spectrometer (MS). This method can be used in conjunction with HPLC or gas chromatography (GC) to determine the amount of acrolein in the sample.

6.2 ENVIRONMENTAL SAMPLES

Acrolein can be determined in air samples using NIOSH method 2501 (NIOSH 1984). In this method, a known volume of air is pumped through a tube containing a support coated with the derivatizing agent 2-(hydroxymethyl)piperidine. The derivative is eluted from the tube with toluene, and analyzed by GC using a nitrogen specific detector (NSD). Variations of this procedure have also been reported. Rietz (1985) used DNP as a derivatizing agent on the adsorbent tube, and made a final analysis using HPLC coupled to a UV detector. Acrolein has also been trapped for analysis by bubbling air through an aliquot of ethanol, adding methoxylamine hydrochloride to form a derivative, and then brominating the resulting adduct to allow increased detector sensitivity. Quantitation is achieved by GC using an electron capture detector (ECD) (Nishikawa et al. 1986). A summary of these techniques, along with methods for other environmental samples, are presented in Table 6-l. Interferences due to coincidental elution of derivatives of the compounds is a potential problem of these techniques.

Derivatization methods for the measurement of acrolein levels in environmental media should be used with caution based upon data for analysis for acrolein in aqueous solutions (Kisel et al. 1978). In a comparison of chemical analytical methods to bioassays, results obtained using methods utilizing derivatives (DNP and 7-hydroxyquinoline) combined with calorimetric or fluorimetric detection were not specific for acrolein and consistently did not correlate with those obtained from bioassays. Certain direct methods of detection (nuclear magnetic resonance (NMR), fluorescence, and differential pulse polarography) gave the best correlation to the bioassay results (see Table 6-l).

The analysis of acrolein in wastewater and water-miscible wastes or soils with low levels of contamination can be performed using EPA methods 603 and 8030, respectively (EPA 1982a, 1986b). In closely related techniques, an aliquot of water is subjected to a purge and trap protocol, and the sample is thermally desorbed onto a GC for analysis and quantitation. For waste samples not miscible with water or for soil samples with high levels of contamination, the sample can first be extracted with ethanol. The extract is diluted with water and then subjected to the purge and trap analysis described above (EPA 1986b). Coincidental elution of compounds with acrolein may lead to interferences in these methods. Ogawa and Fritz (1985) developed a method for the identification of acrolein in water. A known volume of water is passed over a column of

TABLE 6-1. Analytical Methods for Determining Acrolein in Environmental Samples

Method/Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy[a]	Reference
NIOSH 2501/Air	Collection on tube with 2-(hydroxymethyl)piperidine[b] coated on XAD-2 resin, desorbed by toluene extraction.	GC - NSD[c]	2 µg	NS	NIOSH 1984
EPA 603/Wastewater[d]	Purge at 85°C and trap onto methyl silicone/2,6-diphenylene oxide adsorbent, thermal desorption.	GC - FID	0.6 µg/L	96%	EPA 1982a
EPA 8030/Solid Waste[e]	Purge and trap onto adsorbent, rapid adsorbent, rapid heating desorption	GC - FID	NS	NS	EPA 1986
Air	Trap in ethanol solution, add methoxylaminehydrochloride[b], brominate.	GC - ECD[c]	<4 ppb	81-96%	Nishikawa et al. 1986
	Trap on XAD-2 resin coated with 2-(hydroxymethyl)piperidine[b], desorb with toluene.	GC - NSD[c]	NS	NS	Kennedy et al. 1984
	Trap on XAD-2 resin coated with 2,4-DNP[b], elute adduct with acetonitrile.	HPLC - UV[c]	0.01 mg/m^3	5 mg/m^3	Rietz 1985
Water	Trap on Zeolite ZSM-5 column, elute with acetonitrile, derivatize with 2,4-DNP.	HPLC - UV[c]	<10 µg	98%	Ogawa and Fritz 1985
	Nondirect measurement of aldehyde signal compared to signal for a calibrated sealed external TMS standard.	NMR	5000 ppm	NS	Kissel et al. 1978
	Dilution of sample with deionized water.	Fluorescence spectrometer	>20 ppm	NS	Kissel et al. 1978
	Dilution of sample with deionized water, addition of phosphate buffer and EDTA.	Differential pulse polarography	>30 ppb	NS	Kissel et al. 1978

TABLE 6-1 (Continued)

Method/Sample Matrix	Sample Preparation	Analytical Method	Detection Limit	Accuracy[a]	Reference
Personal Air	Trap on carbon coated with hydroquinone, desorb with 1,2-dichloroethane.	GC	0.02 ppb	>75%	Hurley and Ketcham 1978
Rain	Add to collected sample methoxylaminehydrochloride[b], brominate.	GC - ECD[c]	0.4 ng/mL	90-101%	Nishikawa et al. 1987b

[a]Defined as the percent recovery of a blank sample.
[b]Derivatizing agent.
[c]Derivative analyzed.
[d]EPA method 603 is the preferred method for quantitative analysis; method 624 can be used to screen samples for acrolein.
[e]Incorporates EPA method 5030.

2,4-DNP = 2,4-dinitrophenylhydrazine; ECD = electron capture detector; EDTA = ethylenediaminetetraacetic acid; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; NMR = nuclear magnetic resonance; NS = not stated; NSD = nitrogen specific detector; TMS = tetramethylsilane; UV = ultraviolet.

zeolite that traps the acrolein. The column is then eluted with acetonitrile, and derivatization using DNP follows. By following this procedure, a sample that can be analyzed by HPLC is obtained. Other derivatizing agents that have been used successfully for the monitoring of acrolein in the environment include dimedon, phenylhydrazone, 4-hexylresorcinol, and 3-methyl-2-benzothiazolone (Altshuller and McPherson 1963; Peltonen et al. 1984).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of acrolein is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of acrolein.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that, if met would reduce or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No biomarker that can be associated quantitatively with exposure of acrolein has been identified (see Section 2.5). There are methods that can detect acrolein as well as its metabolite 3-hydroxypropylmercapturic acid. The methods used for the analysis of acrolein, however, can be susceptible to interferences. Methods that positively identify acrolein or one of its derivatives would eliminate the problems associated with specificity of the technique.

No biomarker that can be associated quantitatively with effect has been identified (see Section 2.5). Thus, there are no analytical methods for the determination of biomarkers of effect for acrolein.

Methods for Parent Compound and Degradation Products in Environmental Media. Suitable methods for the determination of acrolein in environmental samples exist. Nevertheless, new methodologies for the determination of acrolein have been reported. These methods can offer increased sensitivity and greater ease of performance. Further testing and evaluation of these methods would be useful in establishing the most powerful and practical techniques for routine analysis of acrolein in environmental samples.

6.3.2 On-going Studies

No on-going studies concerning the determination of acrolein in environmental media or biological materials were identified.